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Quantitative detection of atropine-delayed gastric emptying in the horse by the ^{13}C-octanoic acid breath test

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Summary

The ^{13}C-octanoic acid breath test has been correlated significantly to radioscintigraphy for measurement of gastric emptying indices in healthy horses. The objective of this study was to investigate the validity of the test for measurement of equine delayed gastric emptying, prior to its potential clinical application for this purpose. A model of atropine-induced gastroparesis was used. Gastric emptying rate was measured twice in 8 horses using concurrent radioscintigraphy and/or breath test after treatment i.v. with either atropine (0.035 mg/kg bwt) or saline in randomised order.

Analysis of both data sets demonstrated that the atropine treatment had caused a significant delay in gastric emptying rate. Paired breath test data showed an atropine-induced delay in gastric half-emptying time ($t_{1/2}$), with no overlap in the 99% CI range ($P<0.001$). Significant correlations were found between scintigraphy and ^{13}C-octanoic acid breath test for calculation of both $t_{1/2}$ ($P<0.01$) and lag phase duration ($P<0.05$) in the atropine-delayed emptying results. The mean (s.d.) bias in breath test $t_{1/2}$ when compared with scintigraphy was 1.78 (0.58) h.

The results demonstrated that the ^{13}C-octanoic acid breath test was an effective diagnostic modality for the measurement of equine delayed gastric emptying. The technique offers advantages to existing methods for clinical investigation, as it is noninvasive, not radioactive, quantitative and requires minimal equipment or training to perform.

Introduction

Delayed gastric emptying may be involved in the pathogenesis of many important conditions in the horse, such as gastric ulceration, equine dysautonomia, postoperative ileus, gastric impaction and idiopathic recurrent colic, and can be difficult to diagnose clinically. A safe, noninvasive, quantitative test for the measurement of this parameter, that could be used repeatedly in clinical cases, would be of great benefit in equine gastroenterology (Merritt 1997). Previous methods that have been used to assess solid phase gastric emptying in the horse have not given direct physiological information, being based on transit measurements of plastic beads (Adams and MacHarg 1985) or radiographic tracking of radiopaque markers (Baker and Gerring 1994). Gastric radioscintigraphy following intubation with radiolabelled solids (Sojka and Cantwell 1988; Levy and Sojka 1991; Neuwirth 1994; Ringger *et al.* 1996) is considered to be the optimum diagnostic technique, but has the disadvantages of radiation exposure and the requirement for expensive equipment and user expertise. Acetaminophen absorption has been validated in the horse for the measurement of liquid phase emptying, using radioscintigraphy (Lohmann *et al.* 2000). However, solids and liquids empty from the stomach by different mechanisms and rates (Parkman *et al.* 1995) and measurement of solid phase emptying is preferable for clinical purposes.

The ^{13}C-octanoic acid breath test (^{13}C-OABT) was recently validated for the measurement of equine solid phase gastric emptying (GE) using radioscintigraphy (Sutton *et al.* 2002a). This stable isotope breath test was found to be accurate for GE measurement in healthy horses. Following ingestion of a ^{13}C-octanoate labelled meal, the ^{13}C tracer leaves the stomach without being metabolised. After entry of the chyme into the small intestine, the tracer is then absorbed rapidly and undergoes hepatic oxidation, leading to production of $^{13}CO_2$, which is exhaled after exchange with the bicarbonate pool (Bach and Babayan 1982). Since these postgastric events are constant in nature, the rate of change of the $^{13}CO_2$:$^{12}CO_2$ ratio in expiratory breath provides an indirect measure of solid phase GE, as this is the rate-limiting stage (Ghoos *et al.* 1993).

The ^{13}C-OABT has also been validated using radioscintigraphy for the measurement of gastric emptying of solids in man (Ghoos *et al.* 1993; Duan *et al.* 1995; Ziegler *et al.* 1996; Choi *et al.* 1998; Delbende *et al.* 1998) and is an important diagnostic tool for disorders of the upper gastrointestinal tract in preterm and young children (Veereman-Wauters *et al.* 1996; van den Driessche *et al.* 1999), adults and critical care patients (Ritz *et al.* 2001).

The specific objective of this research was to validate the ^{13}C-OABT against radioscintigraphy in horses with significantly delayed gastric emptying, using a model of atropine-induced

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gastroparesis. This validation was necessary in order to establish accuracy of the test prior to diagnostic use in equine clinical cases for quantitative diagnosis of gastroparesis.

Materials and methods

Subjects

Eight mature horses (5 Quarter Horses, 3 Thoroughbreds) from the Texas A&M University research herd were used in this study, with a median age of 11.5 years (mean 13.3, range 7–25 years) and median bodyweight 520.0 kg (mean 507.4, range 424.5–530.0 kg). These animals had no historical or physical evidence of gastrointestinal disease and biochemical and haematological parameters lay within the reference ranges. All subjects were maintained outdoors on a low ^{13}C diet of *ad libitum* alfalfa hay only, for at least 2 weeks prior to each experiment, to ensure constant basal metabolic production of $^{13}CO_2$.

Study design and protocol

Gastric emptying rate of a standard test meal was measured twice in each horse, after randomised treatment with either atropine or saline. The i.v. bolus of saline or atropine was administered via a preplaced jugular catheter, immediately after ingestion of the test meal was completed. A ^{13}C-OABT was performed on each occasion, together with a concurrent radioscintigraphic study when GE was delayed. Paired tests were separated by a period of at least 7 days. A standard atropine dose of 0.035 mg/kg i.v. was used to induce gastroparesis. This dosage was determined by a pilot study, in which the minimum standard dose of atropine necessary to produce a significant delay in GE without causing apparent abdominal pain was investigated. Analysis of reference scintigraphic data collected by the authors from the same population showed that it would be necessary in the present study to extend scintigraphic $t_{1/2}$ to approximately 3.2 h (mean + $t_{0.005}$(s.d.)) in order to delay emptying rate significantly. In the pilot study, a dose of 0.025 mg/kg atropine was insufficient to cause this delay, but the higher dose of 0.035 mg/kg i.v. was found to be effective.

The study was designed to allow paired comparison of gastric function data after randomised saline or atropine administration, using the ^{13}C-OABT. The design also allowed correlation of the breath test technique with scintigraphy for the measurement of GE in horses known to have a significant delay in emptying rate. The study was approved by the Texas A&M University Laboratory Animal Care Committee (Animal Use Protocol 2001-35).

Test meal composition

The test meal consisted of 150 g crimped oats, 100 g bran, 200 ml water and 2 dual-labelled egg yolks. For the combined test, approximately 1 mg/kg ^{13}C-octanoic acid (Octanoic acid-1-^{13}C, minimum 99 atom % ^{13}C)[1] and 20 mCi ^{99m}technetium sulphur colloid (Sulphur Colloid)[2] were added to 2 egg yolks, which were baked in a microwave oven until firm and thoroughly mixed into the test meal. The radiopharmaceutical was omitted when the breath test alone was performed. Food was withdrawn 14 h before the start of the test to ensure that the stomach was empty.

^{13}C-octanoic acid breath test

Expiratory air was sampled using a modified Aeromask[3] and 250 ml Quintron[4] breath collection bag with a unidirectional valve. This technique was rapid and well tolerated, and has been validated previously (Sutton *et al.* 2002a). Breath samples were stored in duplicate in 10 ml Exetainer[5] tubes, prior to stable isotope analysis. Three basal breath samples were collected 60, 15 and 0 min before test meal ingestion (-60, -15, 0 min), and thereafter at 15 min intervals for 8 h, then 30 min intervals for a further 4 h.

Measurement of sample $^{13}CO_2$ content: A continuous flow isotope ratio mass spectrometer (IRMS; PDZ Europa ABCA)[6] was used to measure the total CO_2 content and ^{13}C abundance of each sample, by comparison with a calibrated 5% CO_2 in nitrogen standard gas. Poor expiratory samples of below 0.5% CO_2 were rejected for data analysis. The $^{13}CO_2$:$^{12}CO_2$ ratio of each sample was measured relative to the international limestone standard, and expressed as the $\delta^{13}C$ value: $\delta x = ((Rx - Rs)/Rs) \times 1000$, where Rs and Rx are the ^{13}C:^{12}C atomic ratio of the standard and sample, respectively. Quality control specimens were sampled after each 5 breath samples, with an acceptable s.d. for $\delta^{13}C$ of 0.2 per run. After subtraction of the average ^{13}C-abundance of the 3 baseline (predose) breath samples, the $\delta^{13}C$ ratio of each sample was converted to absolute units (parts per million excess ^{13}C). Data were then expressed as percentage dose recovery (PDR) of the administered isotope per hour. This latter calculation used the formula for resting $\dot{V}CO_2$ described by Orr *et al.* (1975), derived from body mass.

Gastric radioscintigraphy

Each subject was maintained in the nuclear medicine room for the duration of the test and serial left and right gastric scintigraphs were obtained at 15 min intervals, until the radioactive counts/30 s interval in the region of interest had decreased to less than 10% of that at time zero. The first scintigraph was taken immediately after ingestion of the test meal and atropine administration had been completed. Using a dedicated nuclear medicine imaging computer[7], regions of interest were hand-drawn and the total counts in the gastric region recorded for each time point. These counts were decay-corrected to time 0.

Calculation of gastric emptying indices

Radioscintigraphic test data: Counts in the left gastric region of interest were plotted against time as the relative retention of radioactivity after decay correction (Fig 2). The human GE model developed by Siegel *et al.* (1988) was then used to fit a modelled curve to the data, from which indices of GE were calculated:

$$y(t) = 1 - 1(1 - e^{-kt})^{\beta} \quad (1)$$

where $y(t)$ is the fractional meal retention at time t (expressed in hours) and k is the rate of exponential decay of the final phase of the curve i.e. the GE rate per h. β is a rate constant determined by the extrapolated y-intercept of the initial portion of the curve. From this, the scintigraphic lag phase (t_{lags}) prior to exponential GE was calculated. $T_{lags} = \ln \beta/k$ where $\beta > 1$ indicates an initial lag

period prior to emptying and $\beta<1$ indicates rapid early emptying. The scintigraphic half-emptying time ($t_{1/2s}$) was calculated from equation 2:

$$t_{1/2s} = -\ln[1 - 2^{-1/\beta}]/k \quad (2)$$

This value is equal to the time at which the area under the modelled emptying curve demonstrates loss of half of the administered radioactive dose.

^{13}C-OABT data: Data were plotted as PDR/h against time, and once again, indices of gastric emptying were derived using a modelled curve based on human breath test data (Ghoos *et al.* 1993):

$$y = at^b e^{-ct} \quad (3)$$

where y is the percentage of the ^{13}C dose recovered in breath/h; t is time in hours; and a, b and c are regression constants. The breath test gastric half-emptying time, $t_{1/2b}$, is equivalent to the time at which the area under the cumulative ^{13}C recovery curve demonstrates recovery of half the administered isotopic dose. This was calculated using a Microsoft Excel[8] function:

$$t_{1/2b} = \text{Gammainv}(0.5; b + 1; 1/c) \quad (4)$$

For the breath test, the lag phase, t_{lagb}, is equivalent to division of the rate constants: b/c.

Curve fitting and calculation of constants was performed by nonlinear least squares regression analysis using Microsoft Excel Solver[8]. The above models have been shown previously to have good approximation to gastric emptying function in horses. Further information about the modelling techniques is available in the cited references and in Sutton *et al.* (2002a).

Statistical analysis

A paired t test was used to determine the effect of randomised atropine vs. saline administration on mean breath test $t_{1/2b}$. Where assumption requirements were met, 2 sample t tests were used to compare means in independent groups of observations. A Wilcoxon rank sum test was used for this purpose when there was inequality of variance. Significance was set at $P<0.05$ in each case.

Validation of ^{13}C-OABT vs. scintigraphy: The relationships between the GE indices obtained by the 2 diagnostic modalities were evaluated by Pearson correlation and then linear regression. The relationship between $t_{1/2b}$ and $t_{1/2s}$ was investigated for linearity, and for normal distribution and variability of the residuals. The s.e. (mean Y given X) and 95% CI of the regression line were determined using the formula of Bland (1995). Bland Altman statistics were used to evaluate the mean bias between the two techniques for measurement of gastric $t_{1/2}$ (Bland and Altman 1995, 1999) and 95% confidence limits were established.

Validity of the ^{13}C-OABT for detection of delayed gastric emptying: The concurrent scintigraphic and breath test GE data collected in this study was pooled with comparable data from healthy untreated individuals in the same population, which had been collected and reported previously (Sutton *et al.* 2002a). A cut-off point was determined for scintigraphic $t_{1/2s}$ in the untreated group (mean + $t_{0.005}$ (s.d.)) above which $t_{1/2s}$ was described to be delayed. The sensitivity and specificity of the breath test for detection of delayed gastric emptying in the entire data set (n = 21) as compared to the optimum method of scintigraphy was then investigated.

Results

Effect of atropine on gastric emptying rate

The effects of randomised i.v. atropine (0.035 mg/kg) vs. saline administration on exhaled tracer recovery in 8 horses, following ingestion of the ^{13}C-octanoic acid-labelled test meal, are presented in Figure 1. Mean ± s.d. breath test $t_{1/2}$ values for the atropine and saline treatments were 6.76 ± 1.65 and 2.52 ± 0.35 h, respectively. The differences between the paired observations were normally distributed and significant (mean difference = 4.23 ± 1.60 h, n = 8, $P<0.001$). The calculated 99% confidence interval (CI) range for the mean difference excluded zero (2.26 to 6.21 h), confirming that the ^{13}C-OABT was sufficiently sensitive to detect the atropine-induced delay in gastric emptying.

Mean ± s.d. scintigraphic $t_{1/2}$ after atropine in this study was 4.75 ± 1.60 h (n = 8). A Wilcoxon rank sum test showed that this value was significantly delayed relative to directly comparable scintigraphic data collected previously from 11 healthy untreated individuals in the same population ($P<0.01$; Sutton *et al.* 2002a).

Intravenous atropine administration was followed in all individuals by immediate absence or reduction of intestinal borborygmi, tachycardia and pupil dilatation. The tachycardia was used to monitor the effect of the given dose of atropine, with an average return to resting heart rate of approximately 2.5 h. Intestinal borborygmi returned to original frequency and volume approximately 10 h after atropine, and this was similar to the mean time to first defaecation. No overt signs of abdominal discomfort were seen in any individual.

TABLE 1: Gastric emptying indices determined by concurrent radioscintigraphy and ^{13}C-OABT in 8 horses after ingestion of a standard dual-labelled meal, followed by induction of temporary gastroparesis using atropine 0.035 mg/kg bwt

Modality	Parameter	Mean	s.d.	Range	Sample	CV%
Gastric radioscintigraphy	$t_{1/2s}$ (h)	4.75	1.60	2.75–8.12	n = 8	33.68
	t_{lags} (h)	3.64	0.89	2.05–4.12	n = 7	21.98
^{13}C-OABT	$t_{1/2b}$ (h)	6.76	1.65	4.01–9.48	n = 8	24.41
	t_{lagb} (h)	5.63	1.22	3.17–6.91	n = 7	21.67

$t_{1/2s/b}$ = calculated gastric half-emptying time (scintigraphic / breath test); $t_{lags/b}$ = duration of the calculated lag phase (scintigraphic/breath test); CV% = coefficient of interindividual variation.

Correlation between the ^{13}C-OABT and radioscintigraphy for measurement of delayed gastric emptying

A typical example of the results of a combined scintigraphic and ^{13}C-OABT study in one individual with atropine-induced gastroparesis is shown in Figure 2. Both diagnostic modalities revealed a lag phase before apparent passage of the dual-labelled meal into the small intestine. This pattern of isotope movement was seen in 6/8 individuals, with a constant rate of scintigraphic GE after an initial lag phase. In the remaining 2 individuals, GE proceeded slowly from the start of the test without an obvious scintigraphic lag phase, even after a second dose of atropine (total dose of 0.07 mg/kg bwt) was given to these individuals. The mean gastric emptying indices in the 8 subjects after atropine administration are presented in Table 1. Scintigraphic $t_{1/2}$ was calculated in 7/8 individuals using images collected from the left side of the horse. In the remaining individual, it was necessary to use the geometric mean value (based on the square-root of the product of the left and right side counts) due to prolonged activity in the right region of interest; t_{lag} calculations were not included for this horse, due to modelling inaccuracies.

A linear relationship was present between the 2 techniques for calculation of $t_{1/2}$ (Fig 3), with a normal residual distribution, and a Pearson correlation coefficient of 0.867 (95% CI = 0.417–0.976, P<0.01). The 95% upper and lower confidence intervals for the best regression line and its equation (1) are presented in Figure 3. Omitting the outlier seen in Figure 3, the best fit equation is given by equation (2):

$$Y = 1.640 + 1.029x$$

(r = 0.944, 95% CI = 0.663–0.992, n = 7, P<0.001)

For the subjects in which it was calculable (n = 7), a positive correlation was also found between the 2 techniques for measurement of t_{lag} (r = 0.764, P<0.05).

Bland Altman statistics were used to investigate the nature of the mean bias between the techniques for measurement of $t_{1/2}$. After removing the single outlier shown in Figure 3, the mean difference was plotted against average combined value for $t_{1/2s}$ and $t_{1/2b}$, together with the 95% confidence intervals and 95% limits of agreement (Fig 4). The mean bias = 1.78 ± 0.58 h (95% CI = 1.24–2.33 h; 95% limits = 0.63–2.93 h; n = 7). After confirming normal distribution and homoscedasticity of the data, a 2 sample t test showed that the mean difference ($t_{1/2b}$–$t_{1/2s}$) after atropine was not significantly different in this study to that measured previously in 11 individuals from the same population with normal emptying rate.

Validity of ^{13}C-OABT for measurement of delayed gastric emptying

Using comparable, normally distributed scintigraphic data from healthy untreated individuals (n = 11) in the same population (Sutton *et al.* 2002), a cut-off of 3.151 h was calculated below which 99.95% (mean $t_{1/2s}$ + $t_{0.005}$(s.d.)) of population $t_{1/2s}$ would be expected to lie. For the purpose of determining sensitivity and specificity values for breath test data in relation to concurrent scintigraphic data, values for $t_{1/2s}$ above this cut-off were described as 'delayed' GE. Using this scintigraphic cut-off to indicate delayed GE, a breath test $t_{1/2}$ in the range 5.55–5.71 h had a sensitivity of 1.00 for delayed gastric emptying, with a specificity of 0.92, and positive and negative predictive values of 0.89 and 1.00 in an observed prevalence of 0.38 (n = 21). Although based on a small sample, a breath test $t_{1/2}$ of greater than 5.55 h in the combined study populations would be expected to represent delayed GE.

Discussion

In this study, the ^{13}C-octanoic acid breath test was shown to be sufficiently sensitive to detect atropine-induced changes in equine solid phase GE rate. In addition, a significant positive correlation was found between the ^{13}C-OABT and the standard technique of radioscintigraphy for the measurement of gastric $t_{1/2}$ in a population of horses proven to have significantly delayed gastric emptying (n = 8). The mean bias between the tests for calculation of $t_{1/2}$ was not significantly different between this group of horses with delayed emptying and that measured previously in normal

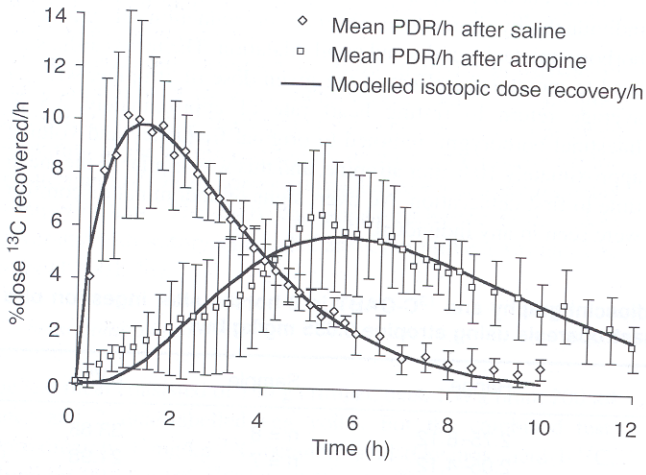

Fig 1: Mean percentage dose recovery (PDR/h) ± s.d. of ^{13}C in the breath after ingestion of the ^{13}C-octanoic acid labelled test meal at time 0, followed by immediate saline or atropine injection in 8 horses. The test was performed twice in each horse (n = 16) in randomised order, separated by an interval of at least one week. Mean saline and atropine $t_{1/2}$ values were significantly different (P<0.001) with no overlap in the 99% C.I. range.

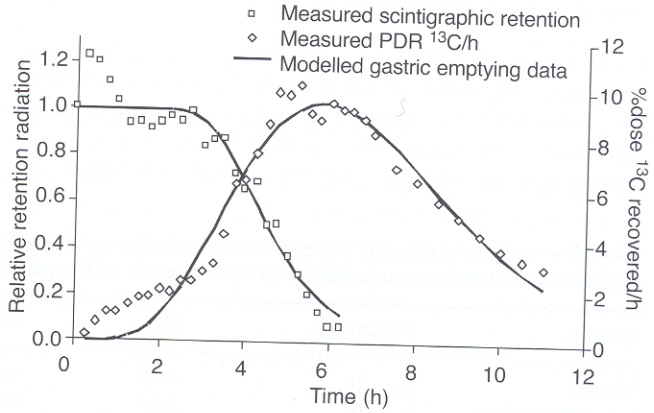

Fig 2: Results of simultaneous ^{13}C-octanoic acid breath test (right y-axis) and gastric radioscintigraphy (left y-axis) in one horse, following intravenous administration of atropine at time 0. Both tests demonstrate a lag phase before movement of the ingested labelled meal into the small intestine. Scintigraphic and breath test $t_{1/2}$ calculated from the modelled curves were 4.72 and 6.01 h, respectively.

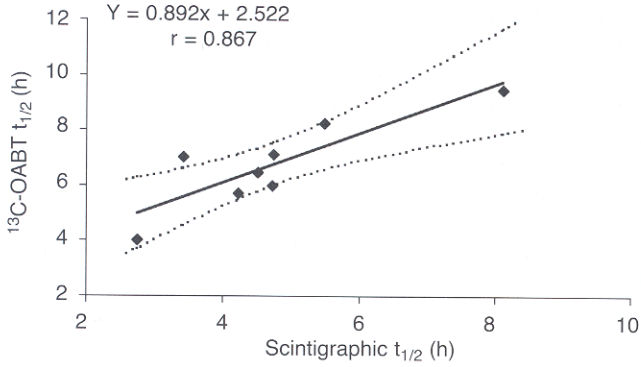

Fig 3: Comparison of gastric half-emptying times ($t_{1/2}$) determined by ^{13}C-octanoic acid breath test with those determined by scintigraphy after atropine administration at time 0 in all individuals (n = 8). The best regression line is shown, and the upper and lower 95% confidence limits are plotted. Pearson correlation coefficient = 0.867, P<0.01.

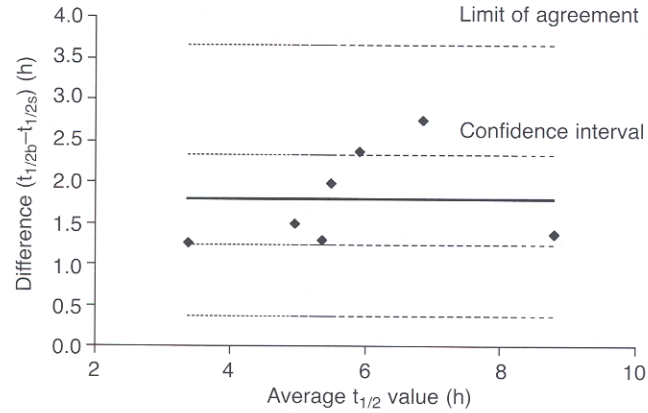

Fig 4: Bland Altman plot showing mean difference in estimate of $t_{1/2}$ for the 2 techniques plotted against average $t_{1/2}$ value. The mean difference (continuous line), 95% confidence intervals and the 95% limits of agreement for the mean bias are plotted. The outlier shown on the linear regression plot has been removed (n = 7).

coexisting individuals (Sutton *et al.* 2002a). These results suggested that the ^{13}C-OABT is a valid diagnostic tool for the measurement of delayed GE of solids, producing accurate quantitative data even when this parameter is significantly prolonged. Using a cut-off value of 5.55 h for $t_{1/2b}$, the breath test had excellent sensitivity, specificity and negative and positive predictive values for delayed gastric emptying in the combined study population when compared to the scintigraphic standard.

The mean bias observed between the 2 diagnostic modalities for calculation of $t_{1/2}$ (1.78 ± 0.58 h) was thought to be due to the postgastric handling of the ^{13}C-octanoic acid. After the rate-limiting GE event has occurred, the medium chain fatty acid must be absorbed and processed by the liver before entry of the ^{13}C tracer into the bicarbonate pool, and expiratory enrichment with $^{13}CO_2$ (Bach and Babayan 1982). Ghoos *et al.* (1993) found $t_{1/2b}$ to have a mean bias of 1.10 h when compared to $t_{1/2s}$ in man. The same researchers found that this delay factor corresponded closely to the half-time for absorption and oxidation of ^{13}C-octanoic acid after direct intraduodenal placement of the tracer (Ghoos *et al.* 1993). Gastroscopic intraduodenal administration of ^{13}C-octanoic acid has also been performed in a small sample of horses (n = 3) and has previously been reported to have an empirical half-time of mean ± s.d. 1.70 ± 0.10 h (Sutton *et al.* 2002a). This latter value supports the theory that the observed lag between the diagnostic modalities for determination of $t_{1/2}$ is due to the postgastric handling of the triglyceride.

Use of a standard correction factor for the ^{13}C-OABT assumes a uniformity for absorption, metabolism and excretion of the tracer between individuals. Although intraindividual reproducibility has been shown to be high, the homogeneity of these factors in man has been questioned (Choi *et al.* 1997). However, the accuracy and reproducibility of the ^{13}C-OABT has been confirmed in several large trials by reference to gastric scintigraphy (Choi *et al.* 1998; Delbende *et al.* 1998). Inter- and intra-individual repeatability of the ^{13}C-OABT in ponies has been shown to be high (Wyse *et al.* 2001). The mean intermodality bias for $t_{1/2}$ reported here was also relatively constant with no discernible effects due to age or body mass. Available data therefore appear to confirm that the technique is valid over a wide range of emptying times for diagnostic use in horses.

In this study, delayed gastric emptying was modelled by the administration of atropine (0.035 mg/kg) after meal consumption in healthy horses. This model has been used widely in both equine (Doherty *et al.* 1998; Lohmann *et al.* 2002) and human medicine (Imbimbo *et al.* 1990; Rashid and Bateman, 1990), although the specific effects of atropine on equine gastric motility have not been reported. Using an acetaminophen absorption test, Doherty *et al.* (1998) reported that 0.025 mg/kg bwt atropine caused a significant delay in liquid gastric emptying rate in ponies. However, this dose was inadequate to delay solid phase emptying in this study, and dosage was increased to 0.035 mg/kg bwt. This dose delayed GE significantly in 6/8 subjects, but a second treatment (0.035 mg/kg bwt) was required in the 2 remaining individuals. Hence, a variation was shown to exist in an individual's sensitivity and gastric response to atropine. Signs of abdominal pain were not seen even at the higher combined dose of 0.07 mg/kg bwt. This was in contrast to previous studies in which doses of 0.044 mg/kg bwt atropine (Ducharme and Fubini 1983) and even topical ophthalmic atropine preparations (Williams *et al.* 2000) have been found to cause abdominal pain in ponies.

Atropine administration caused noteworthy changes to the appearance of the scintigraphic gastric emptying profiles. In all individuals, radioactive counts in the gastric region of interest continued to increase for the first 15 min of the study, before reaching plateau. One such example is seen in Figure 2. This phenomenon was probably due to rapid disruption of oesophageal motility by the atropine, resulting in delayed orogastric transit of residual radioactive ingesta. The muscularis externa of the caudal third of the equine oesophagus comprises smooth rather than striated muscle fibres (Sisson 1975) and manometric studies have shown that normal peristaltic velocity in this region is reduced, and contraction time increased, when compared to the proximal regions (Stick *et al.* 1983; Clark *et al.* 1987). In reported *in vitro* work, atropine effectively abolished the smooth muscle component of oesophageal contraction in guinea pig, rat and human tissue by postganglionic muscarinic-cholinergic blockade (Kerr *et al.* 1995; Halmai *et al.* 1996; Storr *et al.* 2000) and oesophageal transit is significantly slowed in human patients with parasympathetic nerve dysfunction (Cunningham *et al.* 1991). Hence, the contractility of the terminal smooth muscle segment of the equine oesophagus is likely to have been significantly inhibited by the administration of atropine.

In 3/8 cases, left gastric counts continued to increase for 60

min after meal ingestion. In addition to delayed oesophageal transit in these animals, this appeared to result from movement of radioactive ingesta from the right to left gastric regions, perhaps due to increased gastric compliance. Lidums *et al.* (2000) showed that atropine administration in man resulted in increased gastric compliance, with a delay in recovery of postprandial proximal gastric tone and a consequent decrease in motility. Atropine is also known to reduce the postprandial antral manometric motility index (Parkman *et al.* 1999) and this is the main mechanism by which it delays gastric emptying, as coordinated antral pressure activity is required for the trituration of solid food and subsequent expulsion of solids and liquids from the stomach (Becker and Kelly 1983; Camilleri *et al.* 1985). The combination of these factors may have resulted in the movement of radioactive ingesta from distal to proximal stomach in the atropinised horses in this study. Maintenance of canine gastric tone is known to require vagal input (Azpiroz and Malagelada 1987), and the scintigraphic findings in this study provide some evidence that cholinergic input is also involved in the control of equine gastric compliance.

The ^{13}C-OABT is noninvasive, safe and well tolerated by equidae. It is also quantitative, nonsubjective and does not require special skills to perform (Perri *et al.* 1998). Having been validated against radioscintigraphy for the measurement of delayed gastric emptying, it is hoped that this stable isotope breath test will have wide potential research and clinical applications. Use of the test would be beneficial in cases of recurrent and chronic colic (Hillyer and Mair 1997; Mair and Hillyer 1997), particularly in cases of gastric neoplasia (Mair and Hillyer 1991; McKenzie *et al.* 1997), gastric dilatation (Edwards 1993) or pyloric stenosis (Church *et al.* 1986). In addition, quantitative GE data would be helpful in the diagnosis and management of chronic grass sickness cases (Merritt 1997) and of any case requiring prokinetic therapy. Further knowledge of the potential role of disordered gastric emptying in conditions such as gastroduodenal ulceration in both foals (Becht and Byars 1986) and mature individuals (Berschneider *et al.* 1999) may also be beneficial. Currently, the ^{13}C-OABT is being used to investigate the effect of pharmacological agents on equine GE (Sutton *et al.* 2002b) and that of different feeding regimens (Geor *et al.* 2001).

In this study, subjects were maintained on alfalfa hay alone to minimise possible error caused by variation in basal $^{13}CO_2$ abundance in breath. With hindsight, the signal produced by the quantity of ^{13}C-enriched tracer used was sufficient to overcome the requirement to control the natural ^{13}C dietary intake prior to testing. Further collation and analysis of test results should allow the currently time-consuming protocol for sample collection to be optimised, to reduce sampling duration and frequency. Stored samples remain stable for at least 60 days (Schoeller *et al.* 1977) and may be safely sent to diagnostic centres for analysis. The number of commercial and institutional analytical centres for stable isotope breath tests is continuing to increase and dedicated veterinary machines are likely in the near future.

Manufacturers' addresses

[1]Isotec Inc., Benner Road, Miamisburg, Ohio, USA.
[2]CIS-US Inc., Bedford, Massachusetts, USA.
[3]Trudell Medical International, London, Ontario, Canada.
[4]QuinTron Instrument Company, Milwaukee, Wisconsin, USA.
[5]Labco Ltd., High Wycombe, Buckinghamshire, UK.
[6]PDZ Europa Ltd, Sandbach, Cheshire, UK.
[7]Nuclear Mac, Scientific Imaging, Littleton, Colorado, USA.
[8]Microsoft Corporation, One Microsoft Way, Redmond, USA.

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